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Inhibitory Effects of an Aqueous Extract of *Gynura procumbens* on Human Mesangial Cell Proliferation

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Gynura procumbens (Lour.) Merr. has been used in some parts of Southeast Asia as a folk medicine to treat kidney diseases, diabetes mellitus, and hyperlipidemia. The present work was undertaken to prove the mechanisms of G. procumbens in the management of glomerular diseases. We investigated the effect of an aqueous extract of G. procumbens on cell proliferation, DNA synthesis, and the expressions of TGF-β1, PDGF-BB, CDK1, CDK2, and CDK4 in fetal bovine serum-activated human mesangial cells (MCs). The G. procumbens extract inhibited proliferation, DNA synthesis, expressions of PDGF-BB, CDK1, and CDK2 mRNA, and expression of TGF-β1 protein in MCs. The inhibitory effect of G. procumbens on MC proliferation may be mediated by suppression of PDGF-BB and TGF-β1 expressions and the modulation of CDK1 and CDK2 expression. Therefore, G. procumbens shows promise as an adjunct therapy in preventing progressive renal diseases.

Key Words: *Gynura procumbens* (Lour.) Merr., Mesangial cell, Proliferation, Renal disease

INTRODUCTION

Glomerular diseases caused by IgA nephropathy, lupus nephritis, diabetic nephropathy, and glomerulosclerosis are a leading cause of chronic and end-stage renal disease. Depending on the form of glomerular disease, renal function may be lost or deteriorate over time. In response to injury, mesangial cells (MCs), one type of glomerular cells, may undergo several cellular fates, including proliferation, apoptosis, and hypertrophy (Marshall & Shankland, 2006). The proliferation of MCs is a common pathohistological characteristics of glomerulonephritis (Floege & Johnson, 1995; Shankland & Johnson, 1995; Kurogi, 2003), which ultimately leads to renal failure.

In this study, we examined the effectiveness of the extract of *Gynura procumbens* (Lour.) Merr. in ameliorating glomerular diseases. Traditionally, the leaves of *G. procumbens* (Lour.) Merr. have been used as a remedy for kidney diseases, eruptive fevers, rash, hypertension, diabetes mellitus, and hyperlipidemia in some parts of Southeast Asia (Perry, 1980). Despite its long history of clinical use, only a few studies have examined the pharmacological mechanisms of its therapeutic efficacy: *G. procumbens* extract has been shown to reduce serum cholesterol and triglyceride levels in streptozotocin-induced diabetic rats (Zhang & Tan, 2000) and to have anti-inflammatory activity (Iskander et al, 2002). *G. procumbens* extract reduces the systolic blood pressure, serum lactate dehydrogenase, and creatine phosphate kinase levels in spontaneously hypertensive rats (Kim et al, 2006). However, there are no studies on the preservative renal function of *G. procumbens* extract. Therefore, we evaluated the effect of an aqueous extract of *G. procumbens* on cell proliferation, DNA synthesis, and the expression of platelet-derived growth factor (PDGF)-BB, transforming growth factor (TGF)-β1, cyclin-dependent kinase 1 (CDK1), CDK2, and CDK4 in fetal bovine serum (FBS)-activated human MCs.

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METHODS

Plant material

Gynura procumbens (Lour.) Merr. was collected from Karyasari, Jakarta, Indonesia. The taxonomy was confirmed by Professor Irawati, Head of the Botanical Department at the Research Center for Biology, Indonesian Institute of Sciences. A voucher specimen (KU-62001) was preserved at the Department of Pharmacology, College of Medicine, Kyung Hee University, Seoul, Republic of Korea. After air-drying at room temperature and crushing, the leaves of *G. procumbens* (100 g) were added to distilled water and extracted by heating at 120°C for 2 h. The preparation was filtered, concentrated using a rotary evaporator, and lyophilized. The final aqueous extract, weighing 24 g and re

ABBREVIATIONS: PDGF, platelet-derived growth factor; TGF, tansforming growth factor; CDK, cyclin-dependent kinase; MC, mesangial cell.

presenting a yield of 24%, was diluted with saline solution.

Culture of mesangial cells (MCs)

MCs were characterized as described previously (Grandaliano et al, 1999). Normal renal cortex was obtained from a patient undergoing nephrectomy for renal carcinoma. Glomeruli were separated from the cortex by sieving. A primary culture of MCs was established from collagenase-treated glomeruli. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM L-glutamine, 2 mM sodium pyruvate, 1% (v/v) nonessential amino acids, 5 g/ml transferrin, and 5 ng/ml selenium. The culture flasks were kept in an incubator at 5% CO_2 and 37°C. Experiments were performed on the MCs after the third passage..

Cell proliferation

DNA synthesis was measured by the incorporation of ^{3}H-thymidine into trichloroacetic acid (TCA)-insoluble material (Yokozawa et al, 1994). The MCs were seeded in 24-well dishes at a density of 4×10^4 cells/well, grown to confluence, and then starved in serum-free medium for 48 h. To test the suppressive effects of *G. procumbens*, the cells were incubated with an aqueous extract of *G. procumbens* (50 or 100 μg/ml) or 250 μM captopril for 18 h and then activated by 10% FBS for 28 h. During the last 4 h, ^{3}H-thymidine (1 μCi/ml; Amersham, Uppsala, Sweden) was added to the cells. The incorporation of ^{3}H-thymidine was counted in scintillation fluid using a β-counter. In parallel, cell proliferation was measured by direct cell counts as described previously (Terada et al, 1997).

Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR)

To isolate RNA, MCs were plated in 10-cm2 dishes at a density of 1×10^6 cells/dish, grown to confluence, and incubated in serum-free medium for 48 h. The cells were then treated for 18 h with 100 μg/ml *G. procumbens* aqueous extract. After activation with 10% FBS for 2 h, total RNA was isolated using RNA Zol B (Teltest; Friendswood, TX, USA). All experimental samples were reverse transcribed in the same set of experiments, and the efficiency of the reaction was determined with respect to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplification. The sequences of the primers were: *PDGF-BB*, 5'-GAAGGAGCCTGGGTTCCCTG-3' and 5'-TTTCTCACCTGGACAGGTCG-3'; *CDK1*, 5'-TCAAAGCTGGCTCTTGGA-3' and 5'-CCTGGTTTCCATTTGGGA-3'; *CDK2*, 5'-GCTTTCTGCCATTCTCATCG-3' and 5'-GTCCCCAGAGTCCGAAAGAT-3'; *CDK4*, 5'-ACGGGTGTAAGTGCCATCTG-3' and 5'-TGGTGTCGGTGCCTATGGGA-3'; *GAPDH*, 5'-TGGTATCGTGGAAGGACTCATGAC-3' and 5'-ATGCCAGTGAGCTTCCCGTTCAGC-3'. The annealing temperature was fixed at 63°C, and the PCR consisted of 25 cycles for *PDGF-BB*, *CDK4*, and *GAPDH*, and 30 cycles for *CDK1* and *CDK2*. The reaction products were electrophoresed in 2% agarose gels, stained with ethidium bromide, and subjected to computer-assisted densitometry.

TGF-β1 expression assay

To determine TGF-β1 expression in MCs, the concentration of TGF-β1 in cell culture supernatants was measured using a human TGF-β1 ELISA kit (R&D Systems; Minneapolis, MN, USA). Briefly, MCs were plated in 24-well dishes at a density of 4×10^4 cells/well, grown to confluence, and incubated in serum-free medium for 48 h. The cells were then treated for 18 h with 100 μg/ml *G. procumbens* aqueous extract. After activation with 10% FBS for 2 h, the supernatants were harvested. Microplates were coated with anti-recombinant human TGF-β1 antibody, and 200 μl of duplicate activated samples or standard dilutions of TGF-β1 were added, followed by 200 μl of TGF-β1 conjugate, 200 μl of substrate solution, and 50 μl of stop solution, respectively. The absorbance at 450 nm was measured using an ELISA reader.

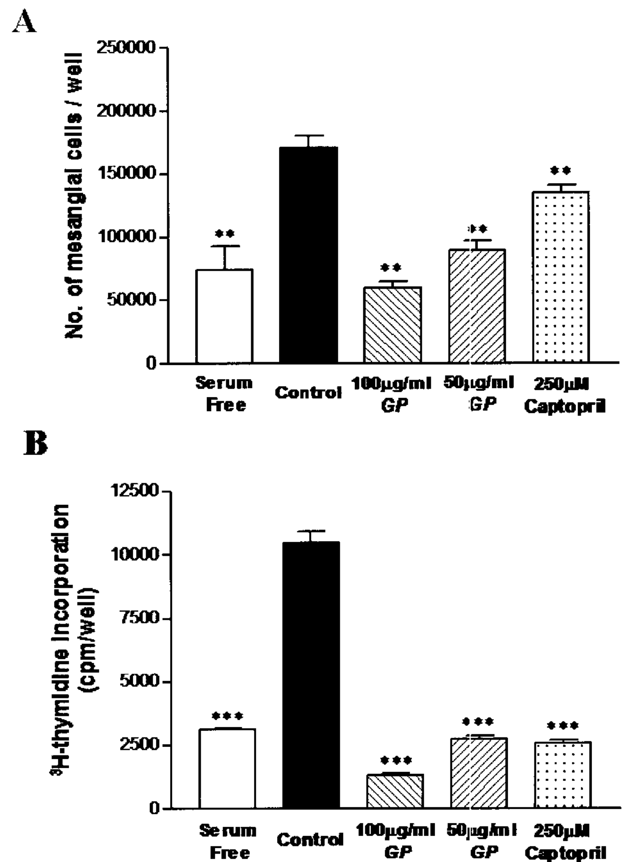

Fig. 1. Effect of aqueous extract of *G. procumbens* on FBS-activated mesangial cell proliferation and DNA synthesis. Mesangial cells were incubated with 100 and 50 μg/ml *G. procumbens* extract and 250 μM captopril for 18 h, and was then activated by 10% FBS for 48 h. (A) The cell proliferation was measured by direct cell counting. (B) The amount of ^{3}H-thymidine incorporation into TCA was measured using β-counter. Results present means±S.D. (n=4). $^{**}p<0.01$, $^{***}p<0.001$ as compared with respective control.

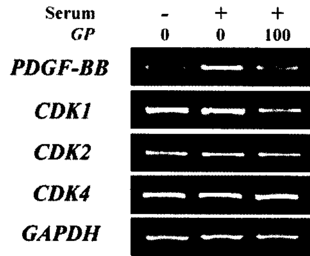

Fig. 2. Inhibitory effect of aqueous extract of *G. procumbens* on FBS-activated *PDGF-BB*, *CDK1* and *CDK2* gene expression. Mesangial cell were incubated with 100 μg/ml aqueous extract of *G. procumbens* for 18 h, and were activated with 10% FBS for 2 h. Cells were collected, and total RNA was isolated and analyzed by RT-PCR for each genes and *GAPDH*.

Statistical analysis

Data are expressed as mean±standard deviation. One-way ANOVA was used to analyze the differences among groups. Values of $p<0.05$ were considered significant.

RESULTS

The anti-proliferative effect of an aqueous extract of *G. procumbens* on FBS-activated human MCs was evaluated by cell counting. In the absence of FBS, the MC count was 74,100±1,900 cells/well. After activation with 10% FBS, the MC density increased to 171,200±8,800 cells/well. The addition of 100 and 50 μg/ml *G. procumbens* extract and 250 μM captopril markedly inhibited MC proliferation in a dose-dependent manner (59,900±5,300, 89,300±7,900, and 135,600±6,200 cells/well, respectively; Fig. 1A). The inhibition of DNA synthesis by *G. procumbens* extract, as measured by ^{3}H-thymidine incorporation, corresponded well with the anti-proliferation effect (Fig. 1B). The aqueous extract of *G. procumbens* at 100 μg/ml showed the inhibition of DNA synthesis greater than 250 μM captopril and the control. Therefore, we used 100 μg/ml aqueous extract of *G. procumbens* to investigate its pharmacological mechanism.

To determine the mechanism of *G. procumbens* action on MC proliferation, we evaluated the expression of the *PDGF-BB*, *CDK1*, *CDK2*, and *CDK4* genes using semi-quantitative RT-PCR and the synthesis of TGF-β1 protein using an ELISA. Compared with the control mRNA transcription level (set at 1), *PDGF-BB* mRNA transcription showed significant inhibition in the presence of 100 μg/ml *G. procumbens* aqueous extract (0.581±0.132; Fig. 2). The transcription of *CDK1* and *CDK2* was reduced to 0.528±0.153 and 0.791±0.156, respectively, in the presence of the extract, whereas *CDK4* mRNA transcription was not significantly changed by the extract (Fig. 2). The aqueous extract of *G. procumbens* significantly inhibited TGF-β1 protein expression (1,370±51.4 pg/ml vs. 1,800±113.2 pg/ml in the control; Fig. 3).

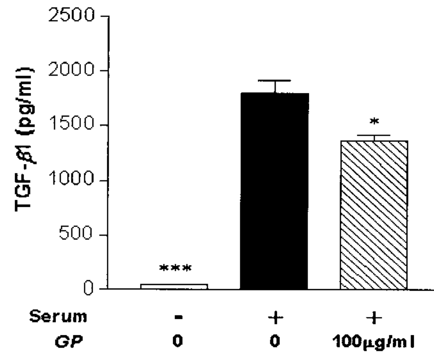

Fig. 3. Inhibitory effect of aqueous extract of *G. procumbens* on FBS-activated TGF-β1 protein expression. Mesangial cells were incubated with 100 μg/ml aqueous extract of *G. procumbens* for 18 h, and were then activated with 10% FBS for 2 h. Supernatant were harvested, and the concentrations of TGF-β1 were measured by human TGF-β1 ELISA assay kit. Results present means±S.D. (n=3). *$p<0.05$, ***$p<0.001$ as compared with respective control.

DISCUSSION

The sequential activation of the cell cycle regulatory proteins (CDKs) underlies the progression of the cell cycle from quiescence through growth to cell division. Several studies have shown that PDGF-BB, TGF-β1, and the CDKs, especially CDK2, are important regulators of MC proliferation (Floege & Johnson, 1995; Shankland & Johnson, 1995; Kurogi, 2003). Increased production of TGF-β1, PDGF, and CDK2 has been observed in association with FBS-stimulated MC proliferation and human IgA nephropathy (Terada et al, 1997; Wang et al, 1998; Kurogi, 2003). Furthermore, the administration of antagonists of *PDGF-BB*, TGF-β1, and CDK2 decreased MC proliferation and glomerulosclerosis in the experimental glomerulonephritic rat (Border et al, 1990; Shankland et al, 1996; Floege et al, 1999). In the present study, we found that *G. procumbens* extract decreased the expression of TGF-β1 protein and *PDGF-BB*, *CDK1*, and *CDK2* mRNA. Captopril is commonly used clinically to reduce not only blood pressure, but also glomerulosclerosis and interstitial fibrosis (Brooks et al, 1993), and to also slow down the progression of renal function deterioration (Praga et al, 1992), especially that related to MC proliferation. Our present results showed that *G. procumbens* inhibits MC proliferation as well as captopril does. Therefore, *G. procumbens* appears to be useful as an adjuvant therapy in patients with chronic glomerulonephritis.

In conclusion, an aqueous extract of *G. procumbens* inhibits MC proliferation and the inhibition may be mediated by the suppression of *PDGF-BB* and TGF-β1 expression and the modulation of *CDK1* and *CDK2* expression. Because of the limitation inherent to crude aqueous extract, further purification to the single compound should be carried out in future. Nevertheless, *G. procumbens* shows promise as an adjunct therapy for the treatment of proliferative glomerular disease.

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REFERENCES

Border WA, Okuda S, Languino LR, Sporn MB, Ruoslahti E. Suppression of experimental glomerulonephritis by antiserum against transforming growth factor beta 1. *Nature* 346: 371–374, 1990

Brooks DP, Short BG, Cyronak MJ, Contino LC, DiCristo M, Wang YX, Ruffolo RR. Comparison between carvedilol and captopril in rats with partial ablation-induced chronic renal failure. *Br J Pharmacol* 109: 581–586, 1993

Floege J, Johnson RJ. Multiple roles for platelet-derived growth factor in renal disease. *Miner Electrolyte Metab* 21: 27–82, 1995

Floege J, Ostendorf T, Janssen U, Burg M, Radeke HH, Vargeese C, Gill SC, Green LS, Janji N. Novel approach to specific growth factor inhibition in vivo: Antagonism of platelet-derived growth factor in glomerulonephritis by aptamers. *Am J Pathol* 154: 169–179, 1999

Grandaliano G, Ranieri E, Monno R, Gesualdo L, Schena F. Ramipril inhibits in vitro human mesangial cell proliferation and platelet-derived growth factor expression. *Exp Nephrol* 7: 229–235, 1999

Iskander MN, Song Y, Coupar IM, Jiratchariyakul W. Antiinflammatory screening of the medicinal plant Gynura procumbens. *Plant Foods Hum Nutr* 57: 234–244, 2002

Kim M, Lee HJ, Wiryowidagdo S, Kim HK. Antihypertensive effects of Gynura procumbens extract in spontaneously hypertensive rats. *J Med Food* 9: 587–590, 2006

Kurogi Y. Mesangial cell proliferation inhibitors for the treatment of proliferative glomerular disease. *Med Res Rev* 23: 15–31, 2003

Marshall CB, Shankland SJ. Cell cycle and glomerular disease: a minireview. *Nephron Exp Nephrol* 102: 39–43, 2006

Perry LM, Medicinal. *Plants of East and Southeast Asia: Attributed Properties and Uses.* The MIT Press, England. p 94–95, 1980

Praga M, Hernandez E, Montoyo C, Andres A, Ruilope LM, Rodicio JL. Long-term beneficial effects of angiotensin-converting enzyme inhibition in patients with nephrotic proteinuria. *Am J Kidney Dis* 20: 240–248, 1992

Shankland SJ, Hugo C, Coats SR, Nangaku M, Pichler RH, Gordon KL, Pippin J, Roberts JM, Couser WG, Johnson RJ. Changes in cell-cycle protein expression during experimental mesangial proliferative glomerulonephritis. *Kidney Int* 50: 1230-1239, 1996

Shankland SJ, Johnson RJ. TGF-β in glomerular disease. *Miner Electrolyte Metab* 24: 168–173, 1995

Terada Y, Yamada T, Nakashima O, Sasaki S, Nonoguchi H, Tomita K, Marumo F. Expression of PDGF and PDGF receptor mRNA in glomeruli in IgA nephropathy. *J Am Soc Nephrol* 8: 817–819, 1997

Wang ZQ, Liang KH, Pahl MV, Vaziri ND. Effect of heparin on mesangial cell growth and gene expression of matrix proteins. *Nephrol Dial Transplant* 13: 3052–3057, 1998

Yokozawa T, Oura H, Hattori M, Iwano M, Dohi K. Effects of Wen-Pi-Tang and its crude drug extracts on proliferation of cultured mouse mesangial cells. *Phytother Res* 8: 170–173, 1994

Zhang XF, Tan BK. Effects of an ethanolic extract of Gynura procumbens on serum glucose, cholesterol and triglyceride levels in normal and streptozotocin-induced diabetic rats. *Singapore Med J* 41: 9–13, 2000